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2. Patent application number
0209640.2

26 APR 2002

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S-112 87
Stockholm
Sweden

Patents ADP number *(if you know it)*

If the applicant is a corporate body, give the country/state of its incorporation

Sweden 6635833002

4. Title of the invention

Height-related Gene

5. Name of your agent *(if you have one)*
"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

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P706097GB

Height-related Gene

The sex-related height difference in humans is thought to be caused mainly by two components: first, a hormonal component determined by the sex dimorphism of bioactive gonadal steroids and second, a genetic component attributed to a Y-specific growth gene, termed GCY (Tanner, *et al.* 1966; Smith, *et al.* 1985; Ogata and Matsuo, 1992). Despite extensive mapping attempts for this gene on the human Y chromosome (Ogata, *et al.* 1995, Salo, *et al.* 1995; Rousseaux-Prevost, *et al.* 1996; De Rosa, *et al.* 1997), its precise position remains unknown. Recent evidence shows that inappropriate cytogenic methodology in the characterisation of Y-chromosomal terminal deletions has brought about some of the difficulties in elucidating the GCY-critical region. In order to overcome these problems, the inventors have considered only patients presenting *de novo* interstitial deletions for the GCY analysis on the Y chromosome (Kirsch, *et al.* 2000). This approach allows the assignment of GCY to a particular chromosomal interval without excluding the presence of XO-mosaicism and/or i(Yp) and idic(Yq11) chromosomes in patients with terminal deletions.

The direct comparison of overlapping interstitial deletions in seven adult males with normal height, one male with borderline height, and one patient with a large interstitial deletion and short stature resulted in the confirmation of the GCY critical interval between markers DYZ3 and DYS11. This region roughly encompasses 1.6-1.7 Mb of genomic DNA. To improve the resolution in the region of interest close to the centromere, the inventors have established additional new STS markers specific for this part of the chromosome using a bacterial artificial chromosome (BAC)/P1-derived artificial chromosome (PAC) contig. Molecular deletion analysis using these new Y-chromosomal STSs allowed the inventors to narrow down the critical interval to a genomic region of 700 kb.

The invention provides an isolated region of the Y chromosome between DYZ3 and DYS11 which encompasses GCY. Preferably the Y chromosome is a human Y chromosome. The region is preferably 700 kb in size.

The invention further provides an isolated gene which contributes the sex related height difference in humans.

Also provided is the GCY protein encoded by a region within the interval between D723 and DYS11.

Further provided is use of a nucleic acid molecule comprising at least a portion of the isolated region of the Y chromasome according to the invention, or a sequence complementary thereto, to identify the presence or absence of a GCY gene.

The invention further encompasses proteins having the same function as GCY protein and which have greater than 65% homology, greater than 70% homology, greater than 75% homology, greater than 80% homology, greater than 85% homology, preferably greater than 90% homology, and most preferably greater than 95% homology to the GCY protein.

Experimental evidence will now be described in detail with reference to the figures in which:

Table 1 is a comparison of the adult height of patients and their siblings.

Table 2 is a table of new Y chromosomal STSs.

Table 3 is the PCR/restriction digest analysis of sequence family variants in the AZFc region.

Table 4 is a summary of BAC and PAC clones identified during physical map creation.

Figure 1. Deletion mapping on the long arm of the human Y chromosome.
A diagram of the human Y chromosome with Yp telomere to the left and Yq telomere to the right is presented at the top. Shown below are the results of low-resolution analysis of Y-chromosomes of adult males with normal height or short stature. [illegible]

all other STSs were previously reported (Vollrath, *et al.* 1992, Jones, *et. al.* 1994, Reijo, *et al.* 1995). Blank spaces or grey boxes indicate inferred absence or presence of markers for which assay was not performed. Asterisks indicate markers in the respective breakpoint regions which could not be tested. In all cases where previously published data of the patients were re-investigated, the identical DNA sample used for the primary analysis was studied. (Please note that the proximal as well as the distal breakpoint of the interstitial deletion of patient #293 resides within satellite type II sequences.)

Figure 2. Sequence family variant (SFV) typing in the human DAZ locus in distal Yq11.23.

A. Overview and amplicon structure of the human Y chromosome in the vicinity of the human DAZ cluster. Each amplicon is represented by a specific colour. Shown above are arrows indicating the orientation of each member of an amplicon family with respect to each other. The amplicon indicated by orange arose from a portion of chromosome 1 that was transposed to the distal end of the DAZ cluster and partially duplicated.

B. Precise position of selected Y-specific STSs and the SFVs according to the physical map of the human Y chromosome. Marker sY157 is highlighted as it was suspected to be present in only one copy by multiplex PCR analysis (see text for detail).

C. Summary of STS and SFV analysis in patients with Y-chromosomal rearrangements within the human DAZ cluster region. Grey boxes indicate inferred absence or presence of markers.

D. Sequence family variant typing of SKY10 and SKY12 in genomic DNA of patient #1972. Assay is described in Table 3. Along the right are listed fragment sizes (in bp). Products are separated by electrophoresis in 3% NuSieve agarose (3:1) and visualized by ethidium bromide staining.

Figure 3. Schematic representation of the organization of the long arm pericentromeric region of the human Y chromosome.

A. Diagram showing the distribution of major tandem repeat blocks and general organization of sequence homologies. Basically, the region can be subdivided in three distinct intervals: a proximal region characterized by 5bp satellite sequences, a central region with high homology to chromosome 1, and a distal region composed of

X/Y-homologous sequences. In the X/Y-homologous region a segment homologous to chromosome Xq25/10q25 has been integrated (Sargent, *et al.* 1999, Wimmer, *et al.* 2001). Below the precise position of the newly established and previously published STS markers in this region are illustrated. At the bottom border, the PAC/BAC contig constructed with the aid of the new STS markers is shown. Prefixes RP1, 5 indicate PAC clones and RP11 BAC clones, respectively.

B. Localization of the GCY critical interval as defined by high-resolution STS mapping in patients with short stature and normal height. Black boindicate the presence, white boxes the absence of the respective STS. Striped boxes depict the dosage unknown regions where the breakpoint resides.

Materials and Methods

Selection of patients

Patients #293, JOLAR, #28, #63 and #95 have been described clinically in detail elsewhere (Skare, *et al.* 1990; Ma, *et al.* 1993; Foresta, *et al.* 1998; Kleiman, *et al.* 1999). Patient Y0308 corresponds to case 1 in the study of Prior, *et al.* 1997. Patients T.M., #1947 and #1972 are phenotypically normal males suffering from idiopathic infertility. Genomic DNA samples were extracted from peripheral blood leukocytes (#28, #63, #95, Y0308, T.M., #1947, #1972) or from lymphoblastoid cell lines (#293, JOLAR). DNA isolated from peripheral blood leukocytes of normal males and females served as internal controls.

Height assessment

As all individuals are of diverse ethnic origins, height was compared to the respective national height standards (Table 1). Patients were of similar age range. When possible, special attention was given to adult height comparisons between parents and siblings. Data are summarised along with the height standard deviation score (SDS) in Table 1. To calculate the SDS, mean adult height and the standard deviation were taken from the corresponding national physical growth studies.

PCR analysis

Reactions were performed in a total volume of 50µl (75mM Tris/HCl pH9.0, 20mM $(NH_4)_2$ SO_4, 0.1%(w/v) Tween20, 1.5mM $MgCl_2$) containing 1.0mM of each oligonucleotide primer, 100ng genomic DNA as template, 5 units of Taq DNA polymerase (Eurogentec), and each dNTP at 1mM in a thermocycler (MJ Research, Inc.) as follows: After an initial denaturation step of 95°C for 5 min., samples were subjected to 30 cycles consisting of 30 sec. at 94°C, 30 sec. at 60°C and 1 min. at 72°C followed by a final extension step of 5 min. at 72°C. The Multiplex PCR was carried out as described in Henegariu, *et al.* 1994 with minor modifications. *Alu-Alu* PCR reactions were essentially carried out as described in Nelson, *et al.* 1991. Amplification products smaller than 1 kb were resolved on 3% NuSieve agarose/ 1% SeaKem GTG agarose (FMC) in 1 x TBE (0.089 M Tris-borate/0.089 M boric acid/20mM EDTA, pH 8.0). For amplification products larger than 1 kb as well as products from *Alu-Alu*-PCR, 1.5% SeaKem GTG agarose gels in 1 x TBE were used for separation.

PCR primers

Y-STSs, loci and PCR conditions have been described previously (Vollrath, *et al.* 1992; Jones, *et al.* 1994; Reijo, *et al.* 1995). Sequences of new Y-chromosomal STSs are listed in Table 2. Y-specific STSs termed SKY wre either derived from YAC, BAC and PAC end sequences or from clone-internal sequences amplified by various combinations of *Alu* primers. Primers for the markers SKY10, 11, 12 and 13 were designed to amplify fragments spanning unique restriction sites within the genomic DAZ locus (SKY10 from RP11-48 K20 (AC024067), RP11-70G12 (AC006983), RP11-141N04 (AC008272), RP11-366C06 (AC015973), RP11-560I18 (AC053522), RP11-175B09 (AL359453), SKY11 and SKY12 from RP11-245K04 (AC007965), RP11-100J21 (AC017005), RP11-506M09 (AC016752),RP11-589P14 (AC025246) and SKY13 from RP11-100J21 (AC017005), RP11-589P14 (AC025246), RP11-823D08 (AC073649), RP11-251M08 (AC010682), RP11-978G18 (AC073893) in order to detect 'sequence family variants' (SFVs).

Restriction analysis of PCR products

PCR products were resolved on agarose gels, the appropriate gel bands cut our and the DNA isolated with GFX™ PCR DNA and Gel Band Purification Kit (Amersham

Pharmacia Biotech, Inc.) according to the manufacturer's protocol. Fragments amplified from SKY5 and SKY6 were digested with TaqI and BsmI, respectively. to detect SFVs at SKY10, SKY11, SKY12 and SKY13, PCR products were digested with restriction enzymes as listed in Table 3.

Sequencing of BAC/PAC/YAC end fragments

DNA from BAC/PAC clones selected for end sequencing were purified with the Nucleobond PC100 Kit (Macherey-Nagel) according to the manufacturer's instructions. End fragments were directly sequenced using the Thermosequenase Fluorescent Labelled Primer Cycle Sequencing Kit (Pharmacia) and analysed on a Pharmacia A.L.F. express (Amersham Pharmacia Biotech). YAC end fragments were generated with Alu/Vector-polymerase chain reaction and subcloned in pCR2.1 with the TOPO-TA cloning Kit (Invitrogen). Sequencing was performed as described.

Fluorescence *in situ* hybridisation

Metaphase spreads were obtained either from primary blood samples or immortalised cell lines. Preparations were made according to standard protocols (Lichter and Cremer, 1992). Cosmid and plasmid DNA was labelled by nick translation with biotin-16-dUTP (La Roche). Slides carrying metaphase spreads were kept in 70% ethanol at 4°C for one week. 200-300ng of labelled plasmid or cosmid DNA, 20-30 μg of human Cot-1 DNA (GIBCO BRL), and hybridisation buffer (50% formamide, 10% dextran sulfate, and 2 x SSC, pH 7.0) were mixed, denatured for 5 min. at 75°C and pre-annealed for 30 min. at 37°C. The slides were denatured for 2 min. in 70% formamide and 2 x SSC, pH7.0, at 72°C (Reid, *et al.* 1992). The pre-annealed probe was hybridised overnight in a humidifying chamber at 37°C. Slides were washed and stained with avidin-conjugated fluorescein isothiocyanate (FITC). The signal was amplified with biotinylated anti-avidin followed by staining with avidin-FITC. For the probe all human telemeres (Oncor) the instructions supplied by the manufacturer were followed. Chromosomes were counterstained with 4',6-diamidino-2-phenylindol dihydrochloride (DAPI). Images were taken separately by using a cooled charge coupled device camera system (Photometrics, Tucson AZ, USA). A Macintosh Quadra 700 was used for camera control and digital image acquisition. The [illegible]

images were recorded for each fluorochrome. Images were overlaid electronically and further processed using the Adobe Photoshop software.

Results

Mapping of interstitial deletions

The inventor studied the DNA of nine adult males who originally consulted reproduction centres about idiopathic infertility, but were otherwise generally healthy. Of the 9 males, 7 were unremarkable with respect to adult height. One patient, #293, with a height of 157cm, presented short stature (SDS -2.9) and one, Y0308, with a height of 167cm showed borderline height, being at the 3rd percentile of normal U.S. height standard (SDS -1.7). Adult height of his parents and siblings are in the normal range (Table 1), his brother being 19cm taller than the patient. Compared to his target height (178cm) and target range (169-187cm) he can be considered short. All men were ascertained solely on the basis of the occurrence of large *de novo* interstitial deletions on the Y chromosome. Only two of those patients had undergone previous chromosomal studies.

In an effort to localise the GCY locus, the inventors focused on that part of the Y chromosome long arm, which was delimited by the boundaries of the interstitial deletions of the patients with short stature (Fig. 1). Recently, a detailed physical map of the human Y chromosome incorporating 758 ordered STSs and 199 completely sequenced BAC clones has been constructed (Tilford, *et al.* 2001). The inventors used a slightly modified PCR multiplex system (Henegariu, *et al.* 1994) to test the absence or presence of 28 DNA loci from the Y chromosome long arm. In patients where sufficient DNA was available for further PCR analysis additional STSs were tested. As a result, 8 of 9 interstitial deletion breakpoints could be positioned (Fig. 1). As the deletions of patients JOLAR, #28, #63, #95, T.M., and #1947, all with normal height, overlap, most of the long arm of the Y chromosome could be excluded as a critical region for GCY.

As the distal breakpoint of the deletion of patient #1972 does not reside within the specific part of the Y chromosome long arm, the nature of the deletion (terminal or interstitial) remained unclear. There was also no overlap of his deletion with the deletions of patients #1947 and T.M. Relying solely on the results obtained by the STS-based interstitial deletion mapping strategy, it was not possible to exclude formally the region distal to sY158 as a potential critical region for GCY. However, multiplex PCR analysis always showed a less intense amplification product for STS sY157 (a Y-derived marker in close vicinity of sY158). To address this problem, the rearranged Y chromosome of patient #1972 was investigated in more detail.

Fluorescence *in situ* hybridisation and sequence family variant typing of patient #1972

The overall integrity of the Y chromosome from patient #1972 was demonstrated by FISH of the cosmids LLOYNC01"M"34F05 (PAR1) and LLOYNC03"M"49B02 (PAR2) as well as the Y-centromere-specific probe Y-97 and the telomere-specific probe 'all human telomeres' (data not shown). Being aware of the complex structural organisation of the human DAZ locus (Fig. 2A), we specifically searched for sequence family variants (SFVs). To prevent misjudging sequence errors as single nucleotide differences, PCR/restriction-digestion assays were developed only from SFVs present in at least two overlapping BAC clones. The localisation of these SFVs is shown in Fig. 2B. As these SFVs could represent allelic variants, ten unrelated normal German males were typed. In all cases, the expected fragment pattern could be detected for the Y-chromosome derived sequences. In contrast, the fragment pattern deduced from the genomic sequence of the chromosome 1-derived BAC clone RP11-560I18 could not be confirmed (see Table 3 for detail). Each SFV-specific PCR/restriction digestion was compared to the presence/absence in the corresponding BAC clones.

Typing the genomic DNA of patient #1972 for all four sequence family variants (SKY10/Tsp509I, SKY11/NlaIII, SKY12/MseI and SKY13/Cac8I + TfiI) revealed the absence of one Y-derived non-allelic sequence variant (Table 3 and Fig. 2C,D). In the case of SKY10 the distal copy if deleted. Not surprisingly, in all other typing experiments the more proximal copy of the respective SFV was detected as well.

Next, we investigated these SFVs in the two patients with the most distal breakpoints (#95 and #1947). Using genomic DNAs, we determined that both non-allelic variants of SKY11, SKY12 and SKY13, and one non-allelic variant of SKY10, were absent in patient #1947, whereas for all tested SFVs one non-allelic variant was absent in patient #95.

Taken together, these results provide evidence that the proximal breakpoint of the interstitial deletion present in the Y chromosome of patient #1972 resides within the interstitial deletion of patient #1947, thereby excluding this genomic region as a potential critical interval for GCY.

Refinement of the GVY critical interval

Based on the molecular analysis of the pericentric region of the long arm of the human Y chromosome (Williams and Tyler-Smith 1997), the physical extension of the GCY critical region as defined by the markers sY78 (DYZ3) and sY83 (DYS11) was estimated to constitute 1.6-1.7 Mb (Fig. 3A) of DNA. The most proximal 400 kb of this region consist exclusively of 5bp satellite sequences separated from the Y centromere only by *Alu* sequences. This constant part of the human Y chromosome is therefore unlikely to contain coding sequences. The remainder of the GCY critical region is composed of X/Y-homologous as well as autsomal/Y-homologous sequence blocks. At the onset of this study, only limited coverage in YAC clones was available for this region. In order to refine the GCY critical interval and to generate gene finding substrates, it was necessary to establish a BAC/PAC-contig of this region.

The inventors generated 25 additional markers, mainly by sequencing the end fragments of BAC, PAC and YAC clones, as well as clone-internal sequences amplified by various combinations of *Alu-Alu* oligonucleotide primer pairs. Of those, only 7 turned out to be Y-specific (SKY1, SKY2 and SKY4-8) (see Table 2 for detail). The BAC and PAC clones identified during the generation of the physical map are summarised in Table 4. Meanwhile, some of these clones have been completely sequenced as they form part of a tiling path for sequencing the human Y chromosome (Tilford, *et al.* 2001). The proximal part of the cloned region between markers sY78 and SKY6 has not been sequenced to date. A selection of clones covering the entire GCY critical region is depicted in Fig. 3.

Confirming the overlap between BAC RP11-295P22 and BAC RP11-322K23 appeared to be the most crucial step in the process of contig construction. Y-specific markers derived from the opposite end fragments of both clones were suspected to amplify identical-sized fragments from two different loci within the same 5bp satellite region. By testing several restriction enzymes known to cut frequently within 5bp satellites composed of the consensus sequence $(TGGAA)_n$, the inventors developed loci-specific PCR/restriction digestion assays. Typing all BAC clones mapping to this sequence block with the appropriate PCR/restriction digestion assay allowed us to precisely position them, thereby confirming their overlaps.

In order to narrow down the critical interval for the GCY gene, the inventors tested for the presence of the newly generated STS in patients #293, Y0308 and JOLAR. These results allowed the inventors to define a small region for the GCY gene (Fig. 3B). Direct sequence comparison showed that the sequenced BAC clones RP11-322K23, RP11-75F05, RP11-461H06, RP11-333E09, RP11-558M10, CITB-298B15 and CITB-203M13 completely cover the mapped region between Y-STSs SKY8 and sY83 (DYS11), suggesting that it encompasses roughly 700 kb.

Discussion

Since the issue on the existence of a Y-specific growth gene (GCY) was first raised, there have been several attempts to define its precise location. Whereas initial studies unanimously pointed towards a common region of the Y chromosome long arm (Salo, *et al.* 1995), more recent investigations have led to the identification of two non-overlapping critical intervals (Rousseaux-Prevost, *et al.* 1996, Ogata, *et al.* 1995, De Rosa, *et al.* 1997). FISH analyses resolved this apparent contradiction by presenting clear evidence that the patient materials used in these initial investigations contained 45,X0 cells and/or i(Yp) or idic (Yq11) chromosomes (Kirsch, *et al.* 2000). Both genetic parameters influence the adult height of a given individual, thereby rendering it impossible to predict whether such

patients have lost GCY or not. Studies with patients carrying *de novo* interstitial deletions are, therefore, much better suited to address the problem of GCY localisation.

In the course of winnowing the literature for patients with small interstitial deletions, in particular close to the centromere, it became clear that those patients are very rare. This prompted the inventors to extend their search for patients carrying large *de novo* interstitial deletions, irrespective of their actual adult height. We examined 9 adult patients, 7 of whom presented normal height. Furthermore, we could show overlapping deletions, thereby excluding GCY to reside between the Y-specific marker DYS11 and the pseudoautosomal region 2 (PAR2). Two patients, #293 and Y0308, presented interstitial deletions enabling the restriction of the GCY critical region to approximately 700 kb of DNA. This region is therefore predicted to harbour one or more genes required for normal human growth.

All 9 patients studied share infertility as a common phenotype, which is in agreement with their large Yq deletions. Despite extensive routine screening of infertile males in reproduction centres, only two patients were found to present borderline/short stature in combination with a confirmed large *de novo* deletion. The inventors therefore conclude that cytogenetically detectable *de novo* deletions enclosing the GCY gene are rare events. It is possible, however, that deletion sizes are generally small in this area and therefore undetectable using the presently available set of markers. In addition, the adult height reduction of 6-8 cm attributed to the Y-specific growth gene (Ogata and Matsuo, 1992) does not necessarily result in the diagnosis of short stature in all affected males. Sex-related adult height difference is determined by the level of bioactive gonadal steroids and the Y-specific growth gene.[1] Parameters such as nutrition, infectious diseases and secular trend are further components influencing the adult height of a given individual. In particular, the mid-parental height contributes to the evaluation of growth reduction.

In summary, the data provided localises GCY to a critical interval marked by the Y-derived markers SKY8 and sY83 (DYS11). this 700 kb interval, recently sequenced by the Human Genome Project (Tilford, *et al.* 2001), does not contain any known gene or any Y-specific ESTs. Different reasons, such as unusual gene structures, e.g. genes consisting of only one

exon, the lack of homology to any identified gene, and spatially or temporally restricted gene expression patterns, could account for this phenomenon.

The human genomic PAC and BAC libraries used in this work were constructed at the RPCI in Buffalo, NY. Clones isolated from these libraries were purchased from the same institution.

Table 1. Adult height comparison of patients and their siblings.

Case	Country of origin	Height of patient (cm) and standard deviation score	National height standard (cm)	Heights of family members (cm) and standard deviation score
#293	USA	157 (SDS -2.9) short	176.9 (SD 6.8)	(F) normal (M) normal (B) normal
Y0308	USA	167 (SDS -1.7) borderline (short?)	176.9 (SD 6.8)	(F) 170 (M) 170 (B) 188 (SDS +1.7) (S) 167 (SDS -0.4)
JOLAR	United Kingdom	168 (SDS -1.0) normal	174.7 (SD 6.7)	(F) normal (M) normal (B) normal
#28	Italy	175 (SDS -0.3) normal	176.7 (SD 6.5)	(F) normal (M) normal
#63	Ethiopia	170 (SDS +0.3) normal	168.0 (SD 7.4)	(F) normal (M) normal
#95	Israel	185 (SDS +1.4) normal	175.6 (SD 6.8)	(F) normal (M) normal
T.M.	Belgium	182 (SDS +1.3) normal	173.5 (SD 6.7)	(F) normal (M) normal
#1947	Germany	175 (SDS -0.8) normal	179.9 (SD 6.4)	(F) normal (M) normal
#1972	Germany	181 (SDS +0.3) normal	179.9 (SD 6.4)	175 (F) 165 (M) 172 (S) (SDS +1.0)

The standard deviation score (SDS) was calculated based on the equation: SDS = (X-M)/SD, where X is an individual's adult height and M and SD are the mean adult height and the ± 1 standard deviation of the normal population, respectively.

(M) mother, (F) father, (S) sister, (B) brother, (NA) not available.

Table 2 Y-chromosomal STSs

STS	Left Primer	Right Primer	Product
SKY1	GGACATTTGGCTGCAGAGAT	TGGCAATGCACTCTCATCAT	255
SKY2	TCAGGACAGACAGGCTGCTA	CCTGCCACTGAGCTCCTTAC	~1700
SKY3	TTCTCCCTCATCTTCCAAGC	GCTTCCATCCATTAGCAAGG	167
SKY4	CCTTTCATTCCATTCTCTTCCA	CGCACTTTATGGACTGCAA	111
SKY5*	CCCTCGTCCATTTCTTTTGA	CCTCGAATTTAATGGATTGC	202
SKY6*	TCAATGGATGCACAGTGTGGC	TCCACTGAATTCCATTGCAC	328
SKY7	GGGAGTGCAAAGGGAAAGAT	CTTTCCATGGGGTGACATTC	223
SKY8	CCATTCATTCGAGTTCATTACG	ATTGGAATGGAATCGGACAG	189
SKY9	GGCCGATGGTCAAACTGTTA	GAAACGGGCTCTGAAATTCT	531
SKY10*	ATAAGGGGCAGGTTTGTCAC	GCTACTTATTCAGTGTTTAACTGACAC	329
SKY11*	AAAGTGGGTGAAGGACATGG	TTTTTGTTTGTGGCAGGTG	469
SKY12*	TTGAGTCACTGGGGATAACTG	TATGGCCCACAATCACTTCA	216
SKY13*	GGCAGCCTAGAAAGTCTTGTTC	CCCTTGGGATTTTGTCTGTT	198

Markers indicated with a * amplify DNA fragments from more than one genomic locus (see Chapter *Restriction analysis of PCR products* for detail).

Table 3 PCR/Restriction Digest Analysis of Sequence Family Variants in the AZFc region

STS	Restriction enzyme	BAC clones	Fragment sizes (bp) after restriction
SKY10	Tsp509I	487K20	279,50
		70G12	329
		560I18	329*
SKY11	NlaIII	245K04	217,154,79,19
		506M09	233,221,15
SKY12	MscI	245K04	88,57,39,32
		506M09	145,39,32
SKY13	CacSI/TfiI	100J21	97,83,23
		589P14	175,23
		251M08	97,50,33,23

*The submitted sequence of the chromosome 1-derived BAC clone RP11-560I18 (AC053522) does not show a Tsp509I restriction site within the genomic fragment amplified by the primer pair SKY10. Restriction analysis of fragments amplified from male and female genomic DNA, from a somatic cell hybrid line containing chromosome 1 as the only chromosome of human origin and from the BAC RP11-560I18 as well shows two fragments of ~180bp and ~155bp indicating a sequence error in the complete sequence of the BAC clone.

Table 4 Summary of BAC and PAC clones identified during physical map creation

Y-STSs	Positive BACs (RPCI11)	Positive PACs (RPCI1, 3-5)
sY83	not screened	83D22
sY82	not screened	83D22, 114A11, 157G08, 966C15
GY8	not screened	114A11, 168E21, 271D03, 635P21, 765H16, 806O15, 904E13, 966C15
sY81	not screened	301P22, 1079J08, 1078C20, 1160A12
14A3C*	not screened	148J07, 1136A14, 1160A12, 1196I23
sY79	75F05, 79E14, 102G24, 322K23, 417D23, 600D11, 612E10, 725I12, 863I08, 903M02, 1125H21	1149H11
SKY1	376B16, 544C11, 544M21	56A05, 85D24, 958M03
SKY2	79P12, 295P22, 376L20, 828O24, 886I11, 910C06	829H08
SKY4	75F05, 322K23, 612E10	not screened
SKY5	174I24, 271E18, 295P22, 588E18, 620I20, 632F11, 684H19, 705O19	not screened
SKY6	174I24, 271E18, 295P22, 588E18, 620I20, 632F11, 684H19, 705O19	not screened

* 14A3C is a hybridization probe previously described by Tyler-Smith et al.1994. It detects a Y-specific HindIII-fragment of 3.5 kb and an additional autosomal fragment.

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Claims

1. An isolated region of the Y chromosome between DY73 and DYS11 which encompasses GCY.

2. An isolated region according to claim 1 in which GCY is in the proximity of DYS11.

3. An isolated region according to claim 1 or claim 2 encompassing GCY, which is 700 kb in size.

4. An isolated region of the Y chromosome according to claims 1 to 3 in which the Y chromosome is a human chromosome.

5. An isolated GCY protein encoded by a region within the interval between DY23 and DYS11.

6. An isolated protein having greater than 65% homology to the GCY protein of claim 3 and which contributes to the sex-related height difference in humans.

7. Use of a nucleic acid molecule comprising at least a portion of the isolated region at the Y chromasome according to the invention, or a sequence complementary thereto, to identify the presence or absence of a GCY gene.

FIGURE 1

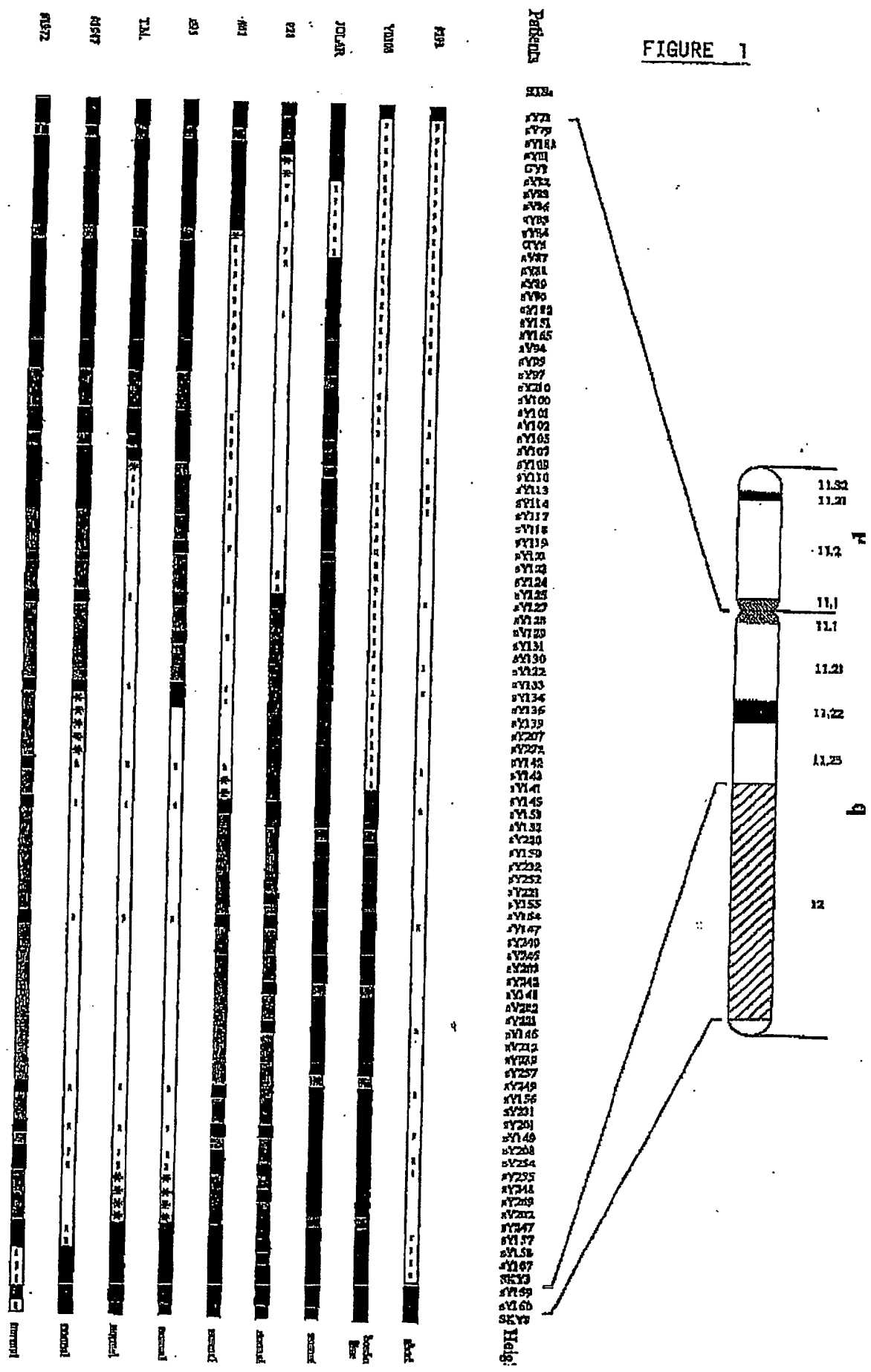
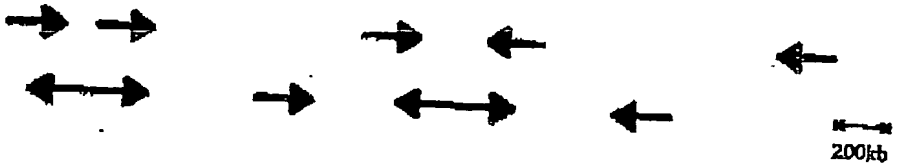
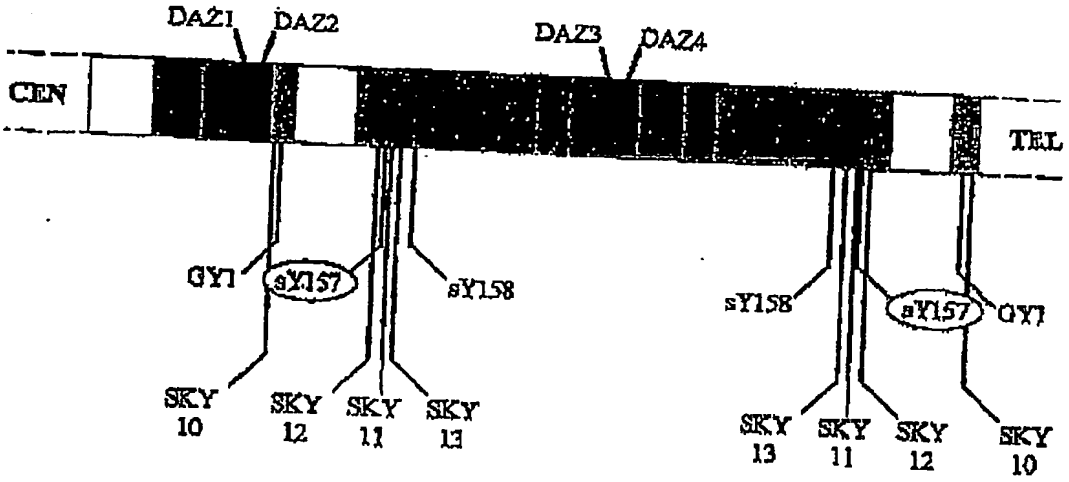


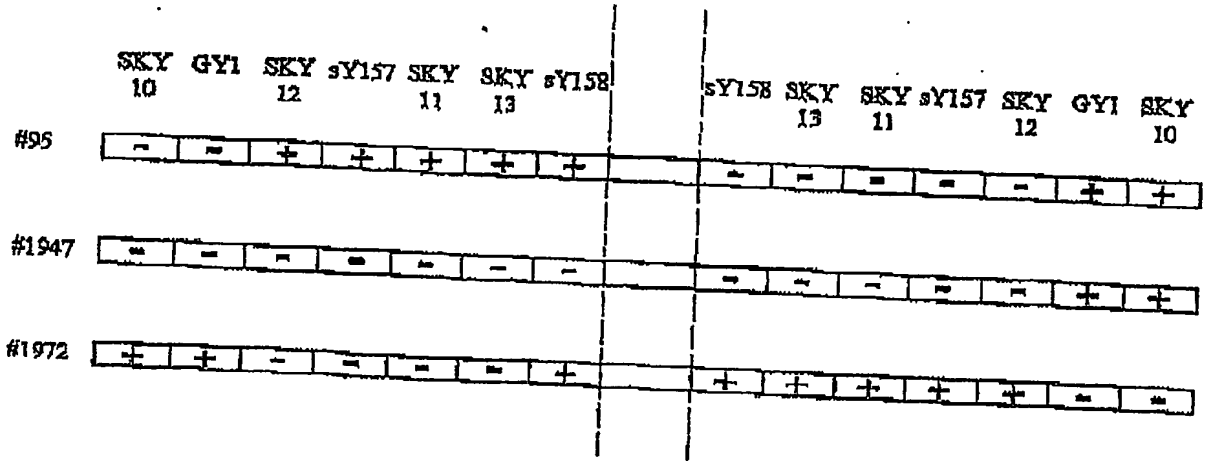
FIGURE 2A



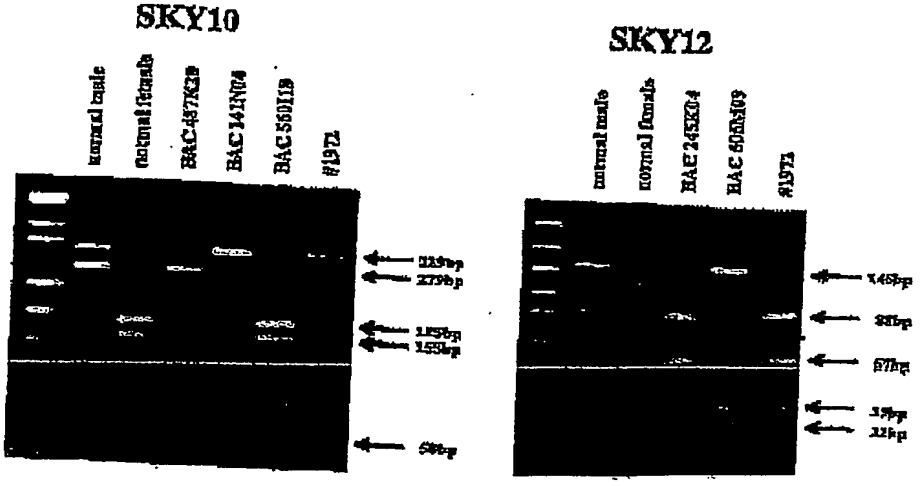
2B



2C

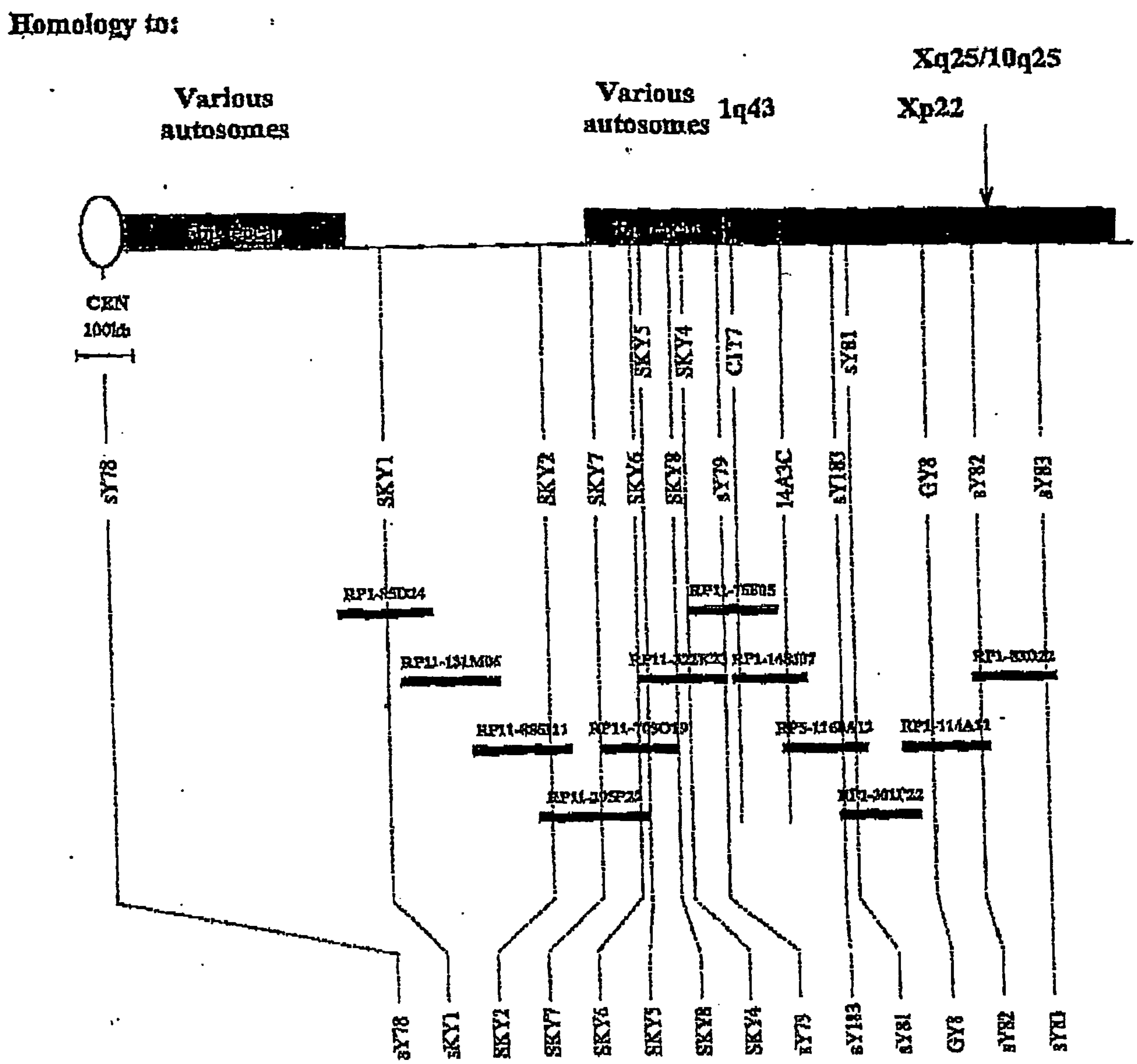


2D



FIGURE

3A


Homology to:
Various autosomes
Various autosomes
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Xp22
Xq25/10q25
CEN
100kb
sY78
SKY1
SKY2
SKY7
SKY6
SKY5
SKY8
SKY4
sY79
C1T7
14A3C
sY183
sY81
GY8
sY82
sY83
sY78
sKY1
SKY2
SKY7
SKY6
SKY5
SKY8
SKY4
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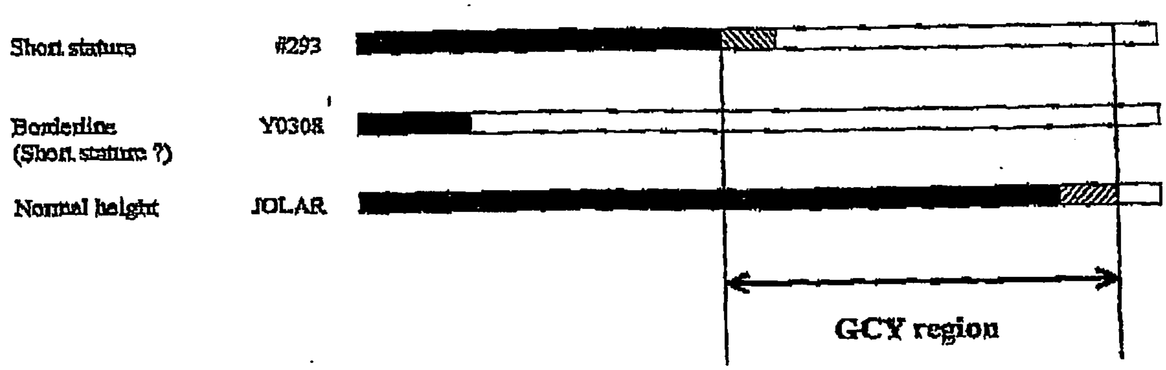
sY183
sY81
GY8
sY82
sY83

3B


Short stature
#293
Borderline
(Short stature ?)
Y0308
Normal height
JOLAR
GCY region